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Electrically conductive gold nanoparticle-chitosan thermosensitive hydrogels for cardiac tissue engineering

Payam Baei [a,b], Sasan Jalili-Firoozinezhad [c,d], Sareh Rajabi-Zeleti [a], Mohammad Tafazzoli-Shadpour [b], Hossein Baharvand [a,e,*], Nasser Aghdami [a,*]

[a] Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
[b] Cardiovascular Engineering Laboratory, Faculty of Biomedical Engineering, Amirkabir University of Technology (Tehran Polytechnic), Tehran, Iran
[c] Department of Biomedicine and Surgery, University Hospital Basel, University of Basel, Hebelstrasse 20, CH-4031 Basel, Switzerland
[d] Department of Bioengineeringand IBB - Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal
[e] Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran

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ABSTRACT

Injectable hydrogels that resemble electromechanical properties of the myocardium are crucial for cardiac tissue engineering prospects. We have developed a facile approach that uses chitosan (CS) to generate a thermosensitive conductive hydrogel with a highly porous network of interconnected pores. Gold nanoparticles (GNPs) were evenly dispersed throughout the CS matrix in order to provide electrical cues. The gelation response and electrical conductivity of the hydrogel were controlled by different concentrations of GNPs. The CS-GNP hydrogels were seeded with mesenchymal stem cells (MSCs) and cultivated for up to 14 days in the absence of electrical stimulations. CS-GNP scaffolds supported viability, metabolism, migration and proliferation of MSCs along with the development of uniform cellular constructs. Immunohistochemistry for early and mature cardiac markers showed enhanced cardiomyogenic differentiation of MSCs within the CS-GNP compared to the CS matrix alone. The results of this study demonstrate that incorporation of nanoscale electro-conductive GNPs into CS hydrogels enhances the properties of myocardial constructs. These constructs could find utilization for regeneration of other electroactive tissues.

1. Introduction

Myocardial infarction (MI) is associated with significant cell death and consequently leads to loss of heart function [1]. So far, cell therapy and various other strategies have been developed to regenerate the structural and functional properties of the injured myocardium. Among them, cardiac tissue engineering offers a promising approach to promote heart tissue repair thanks to the three-dimensional (3D) microenvironment provided by proper scaffolds [2]. Hydrogels have been employed extensively within myocardial engineering facets. In particular, thermoresponsive injectable hydrogel systems have received tremendous attention since they retain the delivered cells in the heart, form gels *in situ* and thereby, obviate invasive surgical procedures [3,4]. Given their thermosensitivity, the hydrogels are liquid in room temperature but once placed in human body (37 °C) via injection, they start solidifying and forming gel structure, and provide proper 3D environment for cellular behaviors. Moreover, bioactive factors (*e.g.* growth factors) could be easily incorporated into the polymer solution, and once exposed to body temperature, these factors are trapped within the gel structure and released in the injected area [5,6]. However, challenges remain in their ability to mimic native mechanical and electrical properties of the myocardium.

Various synthetic and natural materials have been proposed as building blocks of thermosensitive hydrogels to use for cardiac engineering applications [7–9]. Chitosan (CS), an abundant natural product, has attracted increasing attention due to its outstanding biocompatibility, antibacterial and immunologic activity [10–12]. Given the cationic nature of CS, thermoresponsive hydrogels could be readily developed by the simple addition of a polyol salt such as glycerol phosphate [13–15]. Previous studies have aimed to deliver stem cells within the CS hydrogel into the infarcted site with the intent to improve cardiac function [5,16]. Nevertheless, as electrical signals influence a range of cellular activities, CS functionality will be considerably improved if its electrical properties can be enhanced by the introduction of a conductive material.

* Corresponding authors at: Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, P.O. Box 19395-4644, Tehran, Iran
E-mail addresses: Baharvand@royaninstitute.org (H. Baharvand), Nasser.Aghdami@royaninstitute.org (N. Aghdami).

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As one of the major challenges in regeneration of the infarcted heart is the electrical property of the implanted grafts, different approaches have been employed to increase electrical coupling between adjacent cells within the CS scaffolds. These approaches include combination with conducting polymers, *e.g.* polyaniline [17], functionalization with electroactive motifs, *e.g.* aniline oligomer [18], and addition of nanoelectroelements, *e.g.* carbon nanofibers [19]. However, an alternative strategy involves the distribution of gold nanoparticles (GNPs) throughout the construct. GNPs, as biocompatible nanostructures, have the potential to enhance intercellular electrical communications [20]. Their presence in different matrices such as alginate [21], collagen [22] and poly(2-hydroxyethyl methacrylate) [23] has been proven to augment cardiomyocyte function and cardiac differentiation of mesenchymal stem cells (MSCs); while, the interaction of GNPs in the CS scaffold has yet to be studied. The reason of selecting CS lies in the superior behavior of this biomaterial over other previously studied natural polymers. For instance, degradation rate of CS can be simply tuned and estimated by adjusting the degree of deacetylation, to fulfill the required stability matching the growth rate of tissues. Moreover, besides its angiogenic potential in infarcted heart, chitosan with anti-oxidative properties provides a protection against reactive oxygen species and as a result, facilitates the adhesion and homing of cells. Cationic nature of chitosan results in binding of proteoglycans and glycosaminoglycans, which latterly present a proper environment for adhesion of bioactive molecules [5,16,24].

MSCs can potentially differentiate into cardiomyocytes and improve angiogenesis using not only biochemicals such as 5-azacytidine [25], but also biomimetic scaffolds [26]. In this regard, they can be considered a cell source for cardiac engineering prospects. Although signs of cardiac differentiation of MSCs can be observed in scaffolding materials alone, providing electro-conductive cues in their microenvironment can give rise to a cardiac-committed population [27]. In this study, we aimed to generate a novel thermosensitive electro-conductive injectable hydrogel based on CS and GNPs which mimics the electromechanical properties of native myocardium. We sought to examine whether MSCs would maintain their viability, metabolism and proliferation, and enhance their cardiac differentiation within this hydrogel even without electrical stimulation. We developed a facile process to fabricate CS-GNP hydrogels and then checked their adequacy in terms of electromechanical properties and *in vitro* stability. Their effect on MSCs behavior was subsequently examined and the ability of the CS-GNP hydrogel to express specific early and mature cardiac markers was evaluated.

2. Materials and methods

2.1. Characteristics determination of chitosan

The deacetylation degree (DD) of chitosan was determined using potentiometric titration method [28] and based on the following equation:

$$DD\% = \left[\frac{\Delta V \times C_{NaOH} \times 16}{W \times 0.0994}\right] \times 100$$

where:

ΔV is the volume of NaOH between two inflexion points in titration curve (one was the excess HCl and the second was the inflexion point of amino group of chitosan), C_{NaOH} is the concentration of the NaOH solution (0.1 M), 16 g/mol is the molecular weight of the amino group, W is the weight of chitosan sample (50 mg) and 0.0994 is the theoretical percentage of amino groups (no units) in chitosan.

Molecular weight (M_w) of chitosan was obtained using the Mark–Houwink equation: $[\eta] = kM_w^a$, where η is the intrinsic viscosity of chitosan samples derived using an Ubbelohde Viscosimeter obtained at 30 °C and $k = 1.64 \times 10^{-30} \times DD^{14}$, $a = -1.02 \times 10^{-2} \times DD + 1.82$ and DD is the degree of the deacetylation of chitosan expressed as the percentage [29].

2.2. Preparation of chitosan-stabilized gold nanoparticles (CS-GNP)

Tetrachloroauric acid ($HAuCl_4 \cdot 3H_2O$, Sigma, 520918) solutions were prepared in three concentrations, 36, 72 and 108 mM. CS (Sigma, 448877) was dissolved (2%, w/v) in acetic acid (0.1 M, Merck, 100056). $HAuCl_4 \cdot 3H_2O$ and CS solutions were mixed in double distilled water (d.d.H_2O) at 70 °C for 30 min at a molar ratio of 1:1. A red solution that contained GNPs was obtained by the addition of 1 ml sodium citrate (0.1 M, Sigma, C7254) to the resulting solution. The final solution was eventually lyophilized (Christ, Alpha 1–2 LD) to obtain a CS-gold nanoparticle powder (CS-GNP).

2.3. Preparation of a thermoresponsive hydrogel

Both CS and CS-GNP powders were dissolved in 0.1 M acetic acid at constant CS concentrations (2%, w/v). The solutions were subsequently sterilized in an autoclave. β-glycerophosphate disodium salt solution (β-GP, Sigma, G5422, 50% w/v) was prepared in d.d.H_2O, then added dropwise to the CS/CS-GNP solutions for 20 min within an ice-cold water bath. The concentration of CS/CS-GNP and β-GP in final solution was 1.6% and 10% w/v, respectively. This specific molar ratio was selected to keep the gelation behavior close to biological window; moreover, these concentrations have been shown to be cytocompatible [14,30]. The thermoresponsive gel was obtained by placing the resultant solution in a 37 °C incubator. The hydrogels were classified based on the GNP concentration as follows: CS, CS-1GNP, CS-2GNP, and CS-3GNP (Table 1).

2.4. Scanning electron microscopy (SEM)

Morphology and pore features of CS hydrogel were analyzed by field-emission scanning electron microscopy (FESEM, FEI Nova NanoSEM 230, USA). Three lyophilized samples for each group of study were coated with a thin layer of silver (Ag) and observed under an operating voltage of 15 kV. Average pore size of samples was determined using image analyzer software to evaluate 50 images from different sides of each sample (Image J 1.44 p).

2.5. Gelation time

The gelation time for CS and CS-GNP hydrogels was determined by a test-tube-inverting method. Briefly, 1 ml of the prepared solutions was placed in glass vials and incubated in a 37 °C water bath. The gelation point was determined by tilting the tubes and observing the fluidity of samples over 1 min intervals. The time point at which the samples ceased to flow was considered the gelation time. Samples were studied in triplicate and under similar environmental condition.

2.6. Electrical conductivity

The electrical conductivity of samples was measured using a four-point-probe method. Thin films of CS and CS-GNP were prepared by coating these solutions on glass slides. The glass slides were allowed to dry at 37 °C for 24 h. Electrical conductivity of these samples was

Table 1
Summary of the experimental design.

Sample	$HAuCl_4$ concentration (mM)	Final concentration GNP/CS (% w/w)
CS	0	0
CS-1GNP	36	0.5
CS-2GNP	72	1
CS-3GNP	108	1.5

CS: Chitosan; GNP: Gold nanoparticles